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(54) Title: TER SITES AND TER BINDING PROTEINS

(57) Abstract: The present invention provides materials and methods for the utilization of the specific interaction of replication termination sequences with their binding proteins in molecular biology applications.



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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/195; C12N 15/11, 15/64, 15/66, 15/74; C12P 19/34; C12Q 1/68

US CL : 435/6, 91.1, 91.4, 350, 471; 530/350; 536/24.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.1, 91.4, 350, 471; 530/350; 536/24.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DUGGIN et al. Site-directed Mutants of RTP of Bacillus subtilis and the Mechanism of Replication Fork Arrest. Journal of Molecular Biology. 1999, Vol. 286, pages 1325-1335, see entire reference, especially abstract, figure 3, and page 1327, column 2.	1, 3, 7-8, 13-14, 17-19, 34-35, 44-46, 48-49, 54
X	HIDAKA et al. Purification of a DNA Replication Terminus (ter) Site-binding Protein in Eschericia coli and Identification of the Structural Gene. The Journal of Biological Chemistry. 15 December 1989, Vol. 264, No. 35, pages 21031-21037, see entire reference, especially Figs. 4 and 6, page 21031, column 2.	1-4, 17-19, 34-35, 54
X	HILL et al. Identification of the DNA Sequence from the E. coli Terminus Region That Halts Replication Forks. Cell. 4 November 1988, Vol. 55, pages 459-466, see entire reference, especially abstract, page 460, column 2.	20-24, 54
X	NEYLON et al. Interaction of the Eschericia coli Replication Terminator Protein (Tus) with DNA: A Model Derived from DNA-Binding Studies of Mutant Proteins by Surface Plasmon Resonance. Biochemistry. 2000, Vol. 39, pages 11989-11999, see entire reference, especially abstract, Fig. 2, page 11992, column 1.	1, 3, 7-9, 13-14, 17-19, 34-35, 54



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BUSSIERE et al. Termination of DNA replication of bacterial and plasmid chromosomes. Molecular Microbiology. 1999, Vol. 31, No. 6, pages 1611-1618, see entire reference.	1-57
A	GRIFFITHS et al. Replication Terminator Protein-Based Replication Fork-Arrest Systems in Various Bacillus Species. Journal of Bacteriology. July 1998, Vol. 180, No. 13, pages 3360-3367, see entire reference.	1-57

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-6, 20-24, 34-35, and 54, drawn to nucleic acid comprising at least two ter sites or portions thereof, method for directional cloning, and kit.

Group II, claim(s) 7-12, drawn to modified ter binding protein.

Group III, claim(s) 13-16, drawn to solid support comprising at least one oligonucleotide that comprises all or a portion of a ter site.

Group IV, claim(s) 17-19, 25-27, 44-49, and 54, drawn to solid support comprising a ter protein, method for attaching a nucleic acid to a support, and kit.

Group V, claim(s) 28-33, drawn to method of improving the translation efficiency of a nucleic acid.

Group VI, claim(s) 36-38, drawn to method for improving stability of a linear nucleic acid molecule.

Group VII, claim(s) 39-43, drawn to method for deleting a biological molecule.

Group VIII, claim(s) 50-53, drawn to method for separating a nucleic acid containing a ter site from a mixture.

Group IX, claim(s) 55-56, drawn to method of juxtaposing a ter site.

Group X, claim(s) 57, drawn to method of cloning.

The inventions listed as Groups I-X do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

PCT Rule 13.2 requires that unity of invention only exists when there is a shared same or corresponding technical feature among the claimed inventions. The inventions listed as Groups I-IV do not relate to a single general inventive concept because they lack the same or corresponding special technical feature. The "special technical feature" of Group I is a nucleic acid comprising at least two ter sites or portions thereof which is shown by DUGGIN et al to lack novelty or inventive step. The "special technical feature" of Group II is a modified ter protein which is shown by DUGGIN et al to lack novelty or inventive step. The "special technical feature" of Group III is a solid support comprising at least one oligonucleotide comprising at least one ter site or portion thereof which is shown by DUGGIN et al to lack novelty or inventive step. The "special technical feature" of Group IV is a solid support comprising a ter protein which is shown by DUGGIN et al to lack novelty or inventive step.

PCT Rule 13.2 requires that unity of invention only exists when there is a shared same or corresponding technical feature among the claimed inventions. Groups V-X are directed to a method for using one of the products of Groups I-IV, which lack a special technical feature as described above, but each group has a different special technical feature not shared by the remaining groups. Group V is directed to a method which has the special technical feature of contacting a ter-binding protein attached to a support with a nucleic acid which is not shared by any of the remaining groups. Group VI is directed to a method which has the special technical feature of introducing a linear nucleic acid molecule having a ter site bound with a ter protein into a cell which is not shared by any of the remaining groups. Group VII is directed to a method which has the special technical feature of contacting a detection mixture with a nucleic acid binding protein comprising a detection molecule to a nucleic acid molecule bound to a target which is not shared by any of the remaining groups. Group VIII is directed to a method which has the special technical feature of separating a nucleic acid comprising a ter site bound to a ter binding protein from a mixture which is not shared by any of the remaining groups. Group IX is directed to a method which has the special technical feature of causing an enzyme to translocate to juxtapose a ter site and a second site which is not shared by any of the remaining groups. Group X is directed to a method which has the special technical feature of

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ligating a nucleic acid of interest with a vector comprising a portion of a ter site on each end which is not shared by any of the remaining groups.

Continuation of B. FIELDS SEARCHED Item 3:

USPATFUL, PGPUBS, EPO, JPO, Derwent

search terms: replication, arrest, protein, inverted, repeat, region, iri, irii, tus, terminator, terminus, ter, rtp, terb, contra, helicase, termination, replicat?